U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN No. 64.

B. T. GALLOWAY, *Chief of Bureau.*

A METHOD OF DESTROYING OR PREVENTING THE GROWTH OF ALGÆ AND CERTAIN PATHOGENIC BACTERIA IN WATER SUPPLIES.

BY

GEORGE T. MOORE,
PHYSIOLOGIST AND ALGOLOGIST IN CHARGE OF LABORATORY OF PLANT PHYSIOLOGY,

AND

KARL F. KELLERMAN,
ASSISTANT IN PHYSIOLOGY.

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

ISSUED MAY 7, 1904.

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J. E. ROCKWELL, *Editor.*

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
OFFICE OF THE CHIEF,
Washington, D. C., April 30, 1904.

SIR: I have the honor to transmit herewith a paper entitled "A Method of Destroying or Preventing the Growth of Algæ and Certain Pathogenic Bacteria in Water Supplies," and to recommend that it be published as Bulletin No. 64 of the series of this Bureau.

The paper was prepared by George T. Moore, in charge of Laboratory of Plant Physiology, and Karl F. Kellerman, Assistant in Physiology, in the Office of Vegetable Pathological and Physiological Investigations, and was submitted by the Pathologist and Physiologist with a view to publication. The subject discussed in this bulletin will be of interest and value to all who have to deal with the problem of preventing algal and other contamination of water supplies.

Respectfully,

B. T. GALLOWAY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

PREFACE.

The necessity of finding some cheap and practical method of preventing or removing algal contamination of cress beds first led this Office to undertake the investigations described in this bulletin. The success of the first experiments in 1901 was so marked that it seemed wise to extend the work, and authority was, therefore, granted by Congress "to study and find methods for preventing the algal and other contaminations of water supplies."

The progress of the investigation has been noted from time to time in the annual reports of the Bureau. Though the work is not yet completed, we have been urged to publish the results already obtained for the consideration of boards of health and officers in charge of public water supplies.

Doctor Moore and Mr. Kellerman have shown that it is entirely practicable to cheaply and quickly destroy objectionable algæ in small lakes, ponds, storage reservoirs, and other similar bodies of water by the use of extremely dilute solutions of copper sulphate or of metallic copper. The fact that an extremely dilute solution (one to one hundred thousand) will also destroy the most virulent typhoid and cholera bacteria at ordinary temperatures in three hours is of great importance and significance. Solutions of copper as dilute as this are not considered injurious to man or other animals. The value of copper, especially colloidal, in preventing or treating typhoid and other related diseases should be carefully investigated by competent pathologists.

We desire it distinctly understood that, so far as bacterial contamination of water is concerned, the methods here proposed are not to take the place of, but are simply to supplement the standard methods of filtration; neither can too much stress be laid upon the importance of the consumer boiling water to be used for drinking purposes when taken from a contaminated source.

Upon application to the Department by proper authorities, information and assistance will be furnished in determining the organisms causing the trouble in cases of algal pollution, and the proper treatment will be recommended. It is earnestly hoped that no test of the method described here will be made without first consulting the Department.

As stated in the text of the bulletin—

The treatment of water supplies for the destruction of pathogenic bacteria, or any application of the copper sulphate method, which has to do with the public health is not contemplated or indeed possible by this Department. The requests of private individuals or of unauthorized bodies for information or assistance can not be granted. When State or local boards of health consider that the disinfection of a water supply is desirable and wish information upon the subject, it will be supplied as fully and freely as possible. All experiments of this kind, however, must be conducted by boards of health, and the Department can serve only in the capacity of an adviser.

We are under obligation to Dr. H. P. Wolcott and Mr. X. H. Goodnough, of the Massachusetts State Board of Health, for faciiities in securing material and a temporary laboratory in the Boston State House; to the United States Bureau of Fisheries for fish used in experiments; to Dr. J. J. Kinyoun for typhoid cultures; to Dr. M. J. Rosenau for Asiatic cholera cultures, and to the Bureau of Animal Industry for cultures of typhoid and facilities for carrying on preliminary experiments.

ALBERT F. WOODS,
Pathologist and Physiologist.

OFFICE OF VEGETABLE PATHOLOGICAL
AND PHYSIOLOGICAL INVESTIGATIONS,
Washington, D. C., April 30, 1904.

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A METHOD OF DESTROYING OR PREVENTING THE GROWTH OF ALGÆ AND CERTAIN PATHOGENIC BACTERIA IN WATER SUPPLIES.

INTRODUCTION.

The necessity and importance of maintaining by every possible means the purity and wholesomeness of public water supplies have caused those in authority to welcome a method which would in any way serve as an additional safeguard against the pollution of reservoirs or would prevent the bad effects produced by the growth of algæ and similar organisms. Although scientific men have been investigating the various problems involved for a considerable length of time, it is feared that the public has not always been in sympathy with these methods, and that, owing to the uncertainty of and disagreement among eminent authorities, the whole question of water analysis, both chemical and bacteriological, has come somewhat into disrepute.

MICROSCOPICAL EXAMINATION OF DRINKING WATER.

While the best known cases of water pollution are those due to the presence of typhoid and other germs which have given rise to serious epidemics, there are a vastly greater number of water supplies which are rendered unfit for use, not because they are dangerous to public health, but on account of the very offensive odor and taste produced in them by plants other than bacteria. For this reason, in recent years, the question of whether or not a water was fit to drink has been submitted to the biologists as well as to the chemists and bacteriologists, a biological examination being generally understood to mean the determination of the character and quantity of the microscopical plants and animals the water may contain as distinct from the bacteria.

The history of this method of examining drinking water is really confined to the last quarter of the nineteenth century, but only within ten or fifteen years have we had any accurate knowledge of the effect of these minute plants upon the water in which they live. It is probable that Dr. Hassall, of London, was the first to publish any adequate account of a thorough microscopical examination of any water supply, and this work, which appeared in 1850, was practically the only thing

upon the subject for twenty-five years, when "MacDonald's Guide to the Examination of Drinking Water" was published. In the meantime various Germans had carried on investigations relating to the biology of water supplies, notably Professor Cohn, of Breslau, who, in a paper entitled the "Microscopical Analysis of Well Waters," anticipated much that has since been ascertained in regard to the effect of environment upon the character and quantity of the organism found in the water. About the time of the appearance of MacDonald's book, interest in the effect of algæ in drinking water first began to be aroused in this country, and papers by Farlow[a] and others called attention to the fact that these plants were responsible for many of the disagreeable odors and tastes in water reservoirs. By the year 1878 there was on record a list of over 60 cities and towns in the United States which had had serious trouble because of the presence of certain forms of vegetation in their reservoirs, but since then thousands of water supplies throughout the country have been rendered unfit for use by this cause alone. Early in the year 1891 the special report upon the examination and purification of water by the Massachusetts State Board of Health was published, this being the most complete treatment of the subject which had appeared up to that time. This report has been supplemented by further investigations and experiments, and the work accomplished by this board in perfecting methods for insuring a pure water supply has established the standard both in this country and abroad for similar lines of investigation.

WIDE DISTRIBUTION OF TROUBLE CAUSED BY ALGÆ IN WATER SUPPLIES.

In order to demonstrate the very wide distribution of the trouble caused by algæ in water supplies throughout the United States, a circular letter was sent to about five hundred of the leading engineers and superintendents of water companies, asking for information in regard to the deleterious effects produced by plants other than bacteria in water supplies with which they were familiar. Many instructive replies were received, indicating that those in authority were extremely anxious to be provided with some efficient remedy for preventing the bad odors and tastes in drinking water, and that they considered the

[a] FARLOW. Reports on Peculiar Condition of the Water Supplied to the City of Boston. Report of the Cochituate Water Board, 1876.

——— Reports on Matters connected with the Boston Water Supply. Bulletin of Bussey Inst., Jan., 1877.

——— Remarks on Some Algæ found in the Water Supplies of the City of Boston, 1877.

——— On Some Impurities of Drinking Water Caused by Vegetable Growths. Supplement to 1st Ann. Rept. Mass. State Board of Health. Boston, 1880.

——— Relations of Certain Forms of Algæ to Disagreeable Tastes and Odors. Science, II, 333, 1883.

subject worthy of most careful investigation. Quotations from some of the letters received are given, but, because there might be some objection to the naming of towns, only the State in which the trouble occurred is indicated. This is sufficient, however, to show that the difficulty is not confined to any one part of the country, and that it is the algæ alone which are responsible for most of the bad odors and tastes reported.

CALIFORNIA:

Any efforts in the direction of preventing the growth of algæ will be gratefully acknowledged. So long as the growth is healthy it is a benefit, but as soon as the algæ break up then trouble begins.

COLORADO:

We have a reservoir of water that has recently become affected through the presence of micro-organisms of the algæ type that impart to the water a disagreeable fishy odor and render its use objectionable.

DELAWARE:

A fishy taste and odor.

ILLINOIS:

The water tasted and smelled like rotten wood.

Trouble serious enough to cause general complaint by consumers on account of odor and taste.

People declared that the water was musty. The appearance of the growth is yellowish-brown, and as nearly as I can describe it the smell is musty. I certainly think the subject worthy of the best thought and work the Government can give it.

INDIANA:

The growth increased to such an extent that we were compelled to cement the bottom and 5 feet up the sides. It was as dense as a field of clover in June.

Taste was said by the people to be woody or fishy, like rotten wood or decayed fish. At one time the report got out that the body of a missing man had been found in the reservoir.

IOWA:

After certain stages in the alga's growth it seemed to die and become decomposed, thus impregnating the water, giving it a most unpleasant odor and taste.

KENTUCKY:

Fishy odor and taste, rather musty.

The odor was so strong that we had to discontinue sprinkling the streets and lawns.

Urgency in this case is great, indeed almost imperative, since the condition of the water during the past two or three summers has culminated in formal action by the authorities.

MAINE:

Trouble to such an extent as to lead us to consider, without taking definite action, whether or not the water should be filtered before being distributed. Odor is reported as exceedingly disagreeable, so that many customers avoid the use of it as far as possible and believe it injurious to health.

MASSACHUSETTS:

Trouble very serious; some years water is unfit to drink. Present year odor and taste are not so strong as last year, when it was almost impossible to drink it.

The odor was so bad that it would be almost impossible to take it as far as the mouth to taste it. Horses refused it at the street watering troughs and dogs fled from it.

MINNESOTA:

Water at times a fishy odor or taste due to decomposed vegetable matter. Experts claim it is entirely harmless.

NEW JERSEY:

Dark green gelatinous substance in water, causing a stench almost unbearable.

Have seen Uroglena so abundant that an odor could be plainly detected one-third of a mile away.

NEW YORK:

Water had a very fishy taste and smell.

So very offensive as to alarm all water takers.

It caused such a prejudice that the supply was rejected, although the pollution was of short duration.

Strong fishy odor and taste; also odor of "smartweed." Popular complaint was dead fish in water mains.

Odor and taste were fishy, popularly attributed to dead fish; but this is absurd, as the odor is that of live fish.

Odor pondy and fishy; bad water; publicly condemned. Board of health interfered, yet analysis showed that water was not unhealthful.

Very rank, water smelled bad, particularly when warmed. Tasted bad, but not injurious to health. Looked better than tasted or smelled.

Water became unfit for use, musty or cucumber taste and smell, odor very strong in hot water; water became slimy, making it exceedingly hard to filter. Odor and taste at times decidedly fishy. A bright green powder seemed to have been sprinkled on surface.

I am much interested to know that you are taking up an investigation of algæ and organisms, and I very much hope you will favor me with all circulars and information which you may issue relating to the same. I have not attempted to fill out the circular on the back of your letter, but so many cases of trouble of this kind have come to my attention that any listing of them would be very difficult.

I am devoutly thankful that science in this particular instance has got beyond the pursuit of science for recreation's sake and is doing good and endeavoring again directly to be of much use to mankind. I believe your work is the first done in line of either cure or prevention from algæ conducted in a rational manner, or so far as I know even attempted, and I have been connected with or well informed on public water supplies and their management all my professional life of some thirty-five years. The worst case I know of is at the ——— reservoir. A special commission is at this moment charged with the duty of advising whether or not property worth some two million dollars is to be abandoned on account of annual trouble from algæ.

OHIO:

Complaint from customers of a fishy taste in water like the slime from fresh-water fish.

Water had a fishy taste, causing a general kick; consumers laid it to the fish in the reservoir.

All water drawn from house bibbs had an objectionable and strong odor, the popular idea being that it was due to dead fish.

The towns A— and B— both have vile water, A— all the year round, B— for six or eight weeks in the hottest part of the summer. A—'s water has a vile odor, offensively musty. All vegetables, cereals, coffee, and such edibles and drinks made with the water are scarcely endurable to the visitor.

PENNSYLVANIA:

Water had a disagreeable fishy odor.

Water smelled and tasted as if dead fish were in it.

PENNSYLVANIA—Continued.

The growth affected the taste of the water on boiling, but was not regarded as dangerous to health.

A very fishy taste and smell. I have been unable to locate, but had an idea it came from vegetation.

The water during the autumn is so foul in taste and odor that it was necessary to shut off the supply. The odor is similar to that of decayed fish.

The first season of using reservoir the water became so fishy that it was almost unfit for use. Since that, owing to our care of reservoir, we have had no trouble whatever.

TEXAS:

At this time of the year algæ are fierce; some days we are on top and some days the algæ are on top. Costs us an average of $25 a month for cleaning out algæ from two reservoirs.

WISCONSIN:

Universal complaint, caused by the odor and taste due to algæ.

METHODS IN USE FOR PREVENTING BAD EFFECTS DUE TO ALGÆ.

In order to prevent the odors and tastes above described, engineers and those in charge of water supplies have tried various remedies, none of which has been perfectly satisfactory. Since few of the algæ can develop without sunlight, the most frequent recommendation has been to cover the reservoir, and this method has proved successful in a few instances. However, the expense involved is so great as to make the remedy prohibitive in most cases, and other methods have had to be resorted to. One precaution which is now almost universally recommended as a means of preventing the growth of algæ is to remove all the organic matter possible from the reservoir and to keep the source of supply as free as can be from dead and decaying animal and vegetable matter. In one notable instance millions of dollars have been spent in the removal of earth and the substitution of gravel at the bottom of an immense new reservoir. It remains to be seen, however, whether this will be sufficient to insure permanent freedom from these troublesome plants. It is certain that attempts of this kind will delay the appearance of algæ in quantity, and, wherever it is possible to do so, every effort should be made not only to clean up the reservoir at the time of its construction, but to keep it as free as possible from organic matter after it is filled. In addition to cleanliness a direct pumping system with duplicate, in case of breakdown or repairs, has often been recommended for use with ground water, which usually produces a more luxuriant growth of algæ and similar organisms than surface water. Where it has been necessary to store such water, it has been advisable to limit the capacity of the reservoir, and frequently this storage is only intended to be used in case of fire. Even so, the cleansing of the reservoir and the frequent flushing of the water mains has been considered necessary. In storing surface water subdividing the reservoir is occasionally resorted to, and means

of obtaining frequent agitation are introduced wherever possible. The pumping of air into water or aerating it by means of a spraying apparatus is often of considerable value in removing foul gases which may be in solution, but the effect of aeration upon the growth of algæ in a reservoir has been very much overestimated, in some cases the quantity being actually increased by this means.

The filtration of water, both mechanically and by sand, which has proved so effective for the removal of pathogenic bacteria, has been recommended as a means of removing the odors and tastes caused by algæ, but the results obtained have not given promise of success. Perhaps the most careful experiments to determine this point have been conducted by those in charge of the Ludlow reservoir at Springfield, Mass. Here the annual trouble from algæ for the past fifteen years has been so great that every possible means has been used which offered any relief from the effects produced by these plants. On page 4 of the "Special Report on the Improvement of the Present Water Supply and an Alternative New, Independent Supply," made by the board of water commissioners to the city council of the city of Springfield, Mass., April 14, 1902, the following statement is made:

> We find, as the results of the experiments of filtration, made with the sanction of your honorable body during the last fifteen months, that to purify the waters of this source by filtration would be not only doubtful as to the degree of purification, but so expensive in the cost of construction and perpetual maintenance thereafter as to make it inexpedient to attempt improvement by such a method. Your board has given constant and personal attention to the experimental work, and is convinced that the excessive growths of obnoxious fresh-water organisms, notably the Anabaena, impart to the reservoir such rank and persistent tastes and odors as to make uncertain entire removal by any method of filtration except that of the expensive kind, applicable only to the filtering of extremely small quantities of water, and requiring constant attention and adjustment.

The State board of health, in a special report (p. 84) submitted at the same time, say that the results of the experiments indicate, in the opinion of the board, that by double filtration it will be possible to purify the Ludlow reservoir; hence there seem to be differences of opinion as to the value of this treatment for the removal of odors and tastes, but on account of the expense involved there is not likely to be any very extensive use of this method.

DESIRABILITY OF OTHER METHODS.

While each of the above-mentioned methods has been used with some success, it is generally conceded by engineers that there is no known remedy which is universally applicable. It is the practice of some of the highest authorities to recommend that reservoirs frequently polluted by algæ be abandoned, and steps taken to provide an entirely new system of supply. This is, of course, the last resort, as in all such cases a large loss of money is involved. One fact is certain.

If any known method of preventing the growth of algæ was considered truly effective, it would under all circumstances be recommended.

Because of the unsatisfactory results or the prohibitive expense of the present methods recommended for ridding reservoirs of algæ, it seemed advisable that the problem be taken up from an entirely new standpoint, one that would take into consideration the biological aspect of the question and perhaps furnish a solution, through a study of the physiology of the organisms under laboratory conditions. A series of investigations were therefore undertaken to discover, if possible, some substance which, because of its extreme toxic effect upon the algæ involved, would absolutely prevent their growth in water supplies.

DETERMINATION OF A PHYSIOLOGICAL METHOD.

In determining such a physiological method of dealing with reservoirs contaminated by algæ, two conditions had to be considered: The remedy should not only be readily available and cheap enough for practical use in the largest reservoirs and by the poorest communities, but under the conditions used it must also be absolutely harmless to man; the maximum amount necessary to kill the algæ being far below the amount which could in any way affect the consumer of the water. Of the large number of substances experimented with, few gave encouraging results. Free chlorine at a dilution of 1 to 10,000, and sulphur dioxide in saturated aqueous solution at 16° C., diluted 1 to 1,000 and to 10,000, will destroy many of the common forms of algæ, but sulphur dioxide and chlorine are likewise very injurious to animal life. Silver has a very high toxicity, and were not the expense prohibitive, would undoubtedly warrant extended tests. Mercury and lead are, of course, out of the question, and zinc requires too high a concentration to be practically considered. The ordinary sodium, potassium, and ammonium salts are innocuous,[a] as are most of the acids. Loew[b] finds that magnesium sulphate is toxic in pure solution at 0.4 per cent, and that oxalates are slightly more toxic; of the acids, 0.0001 per cent oxalic kills most of the cells of *Spirogyra majuscula* in five days. Migula[c] notes the effect of many of the organic acids, but the use of these substances in the amounts requisite for treating a contaminated water supply is entirely impracticable.

EFFECT OF COPPER SULPHATE.

Reviewing the experiments carried on in the Laboratory of Plant Physiology, as well as the results obtained by other investigators, it

[a] Cf. Richter, Flora, **75**: 4.

[b] Loew, Flora, **75**: 368.

[c] Migula, Ueber den Einfluss stark verduenter Sauren auf Algenzellen, Breslau, 1888. (Original not consulted.)

seems that copper sulphate is the substance best adapted to the work in question. This salt has a very high toxicity for algæ, and experiments with a number of the forms usually found in reservoirs, and the source of much trouble, have shown that inconceivably small amounts of copper are poisonous in a high degree. These experiments demonstrated, however, that all algæ and protozoa are not equally sensitive. Among the latter *Paramæcium* is killed in three hours by a 1 to 1,000,000 solution, while *Amœba*, *Difflugia*, and *Spirostomum* die within two hours. Crustacea are more resistant, some—*Cypris* and *Daphnia* especially—requiring as much as 1 part copper sulphate to 10,000 of water to kill them. Mosquito larvæ die at a concentration varying from 10,000 to 200,000.

Quoting the results of other experimenters, Devaux[a] found that both phænogams and cryptogams were poisoned by solutions of copper diluted to the ten-millionth part or less; Coupin[b] that 1 part copper sulphate to 700,000,000 of water was sufficient to affect the growth of seedlings when applied to their roots and that this is the most injurious of the heavy metal salts tested by him; Deherain and De Moussy[c] that the development of the roots of seedlings was arrested in distilled water containing the slightest trace of copper, and they conclude from this that higher plants during germination, as well as fungi and algæ, are extremely sensitive to copper; Bain's experiments[d] indicated that 1 part of metallic copper to 25,000,000 of water was fatal to apple seedlings in one day; on the other hand, according to Raulin,[e] copper chloride does not injure *Sterigmatocystis* until a concentration of 1 to 240 is reached, although silver nitrate is toxic at 1 to 1,600,000.

In dealing with algæ, the toxic concentration varies greatly for different genera, even for different species in the same genus. Nägeli[f] demonstrated the extreme sensitiveness of *Spirogyra nitida* and *S. dubia* to the presence of copper coins in the water. *Oscillatoria*, *Cladophora*, *Œdogonium*, and the diatoms succumb in six hours to a copper sulphate solution of 1 to 20,000, and in two days to 1 to 50,000, according to Bokorny.[g] Galeotti[h] finds that a concentration between 1 to 6,300,000 and 1 to 12,600,000 is sufficient to kill *Spirogyra nitida* in two days, and that the so-called colloidal solutions at 1 to 6,300,000 are fatal in the same length of time; while in the experi-

[a] Devaux, Compt. Rend., **132**: 717.

[b] Coupin, Compt. Rend., **132**: 645.

[c] Deherain and De Moussy, Compt. Rend., **132**: 523.

[d] Bain, Bull. Agr. Exp. Sta. Tenn., April, 1902.

[e] Raulin, Ann. des Sc. Nat. Bot., 5[e] Ser., II: 93.

[f] Nägeli, Ueber oligodynamische Erscheinungen in lebenden Zellen. Neue Denkschr. d. schweizerischen Gesellsch. für die gesammten Naturwiss., **33**: 51.

[g] Bokorny, Arch. f. d. ges. Phys. d. Mensch. u. Thiere, **64**: 262.

[h] Galeotti, Biol. Centralbl., **21**: 321.

ments of Israel and Klingman[a] the presence of 60 sq. cm. of copper foil in 300 cc. of water for twenty-four hours produced plasmal cutting in *S. laxa* after one and one-fourth hours, in *S. crassa* after fifteen minutes, and in *S. majuscula* after thirty minutes. The work of Rumm[b] shows 1 to 10,000,000 solution still toxic to a few more susceptible cells of *S. longata*. According to Ono,[c] weak solutions of the salts of most of the metals encourage the growth of algæ and fungi. Mercury and copper, however, at 0.00005 per cent and 0.00001 per cent, respectively, distinctly inhibit growth. This was the case with *Stigeoclonium*, *Chroococcum*, and *Protococcus*.

In the experiments conducted in this laboratory it has not been possible as yet to include all of the organisms known to pollute water supplies. It is believed, however, that, pending the completion of more extensive work, the data at hand will be of considerable benefit to those who have to deal with contaminated reservoirs. The method of procedure in studying this question was to determine roughly the death points of the forms under consideration, using Van Tieghem cells. Accurate solutions were then made, with distilled water, and 200 cc. of each solution was pipetted into an Erlenmyer flask. The algæ, if filamentous forms, were rinsed; if free-swimming, they were concentrated by the Sedgwick-Rafter[d] method from 500 cc. to 5 cc. volume, and this 5 cc. was added to the treated water. The inaccuracy due to the addition of the 5 cc. of untreated water to the 200 cc. of treated water was disregarded. Whenever possible, a test of these concentrations, determined experimentally, was made under natural conditions by treating the pool from which the species under consideration was taken. If this was impracticable, an additional series was carried through in aquaria of 15 liters capacity, in which were kept goldfish, frogs, minnows, crustacea, and rotifers. Since in no case was there an appreciable difference in the effect of a concentration upon a particular organism under either natural or artificial conditions, no special record is made of these gross experiments.

The different species tested may, for convenience, be grouped as (1) those with death points at higher concentrations than 1 part copper sulphate to 1,000,000 parts of water; (2) those with death points between 1 to 1,000,000 and 1 to 5,000,000; and (3) those with death points at greater dilutions than 1 to 5,000,000.

[a] Israel and Klingman, Virchow's Archiv., **147**: 293.
[b] Rumm, Beitrage zur Wissenschaftliche Botanik, **1**: 97.
[c] Ono, Journ. of College of Sc., Imp. Univ. Tokyo, **13**: 141.
[d] Whipple, The Microscopy of Drinking Water, New York, 1899, p. 15.

Effect of various concentrations of copper sulphate upon different forms of algæ.

[d=dead; vfa=very few alive; vfd=very few dead; g=in good condition.]

GROUP 1.

CHLAMYDOMONAS PIRIFORMIS Dill.

Date.	One part copper sulphate to water, parts—						Check.
	2,000	5,000	10,000	20,000	200,000	1,000,000	
October 19–21	$\frac{1}{2}$d	g	g	g	g	g	g
October 21–24	$\frac{1}{10}$d	vfd	g	g	g	g	g
October 24–27	$\frac{1}{4}$d	vfd	g	g	g	g	g

RAPHIDIUM POLYMORPHUM Fres.

Date.	One part copper sulphate to water, parts—						Check.
	25,000	50,000	75,000	100,000	500,000	1,000,000	
October 19–29	d	d	$\frac{1}{2}$d	$\frac{1}{10}$d	g	g	g
November 2–6	d	$\frac{3}{4}$d	$\frac{1}{2}$d	$\frac{1}{10}$d	g	g	g
November 16–20	d	vfa	$\frac{1}{2}$d	vfd	g	g	g

DESMIDIUM SWARTZII Ag.

Date.	One part copper sulphate to water, parts—						Check.
	50,000	75,000	100,000	150,000	200,000	1,000,000	
December 2–5	d	d	$\frac{1}{2}$d	vfd	g	g	g
January 4–7	d	d	$\frac{3}{4}$d	vfd	g	g	g

STIGEOCLONIUM TENUE (Ag.) Rabenh.

Date.	One part copper sulphate to water, parts—						Check.
	50,000	100,000	300,000	500,000	1,000,000	2,000,000	
December 21–24	$\frac{1}{2}$d	$\frac{1}{2}$d	$\frac{1}{2}$d	$\frac{1}{2}$d	vfd	g	g
January 2–5	$\frac{1}{2}$d	$\frac{1}{2}$d	$\frac{1}{2}$d	$\frac{1}{2}$d	vfd	g	g
January 7–11	$\frac{1}{2}$d	$\frac{1}{2}$d	$\frac{1}{2}$d	$\frac{1}{2}$d	vfd	g	g

DRAPARNALDIA GLOMERATA (Vauch.) Ag.

Date.	One part copper sulphate to water, parts—						Check.
	50,000	100,000	300,000	500,000	1,000,000	2,000,000	
December 1–8	$\frac{1}{2}$d	$\frac{1}{2}$d	$\frac{1}{4}$d	$\frac{1}{4}$d	vfd	g	g

NAVICULA Sp.

Date.	One part copper sulphate to water, parts—						Check.
	100,000	200,000	300,000	400,000	500,000	1,000,000	
October 20–25	d	d	$\frac{1}{2}$d	vfd	vfd	g	$\frac{1}{2}$d
January 4–9	d	vfa	$\frac{1}{2}$d	vfd	vfd	g	g

Effect of various concentrations of copper sulphate upon different forms of algæ—Cont'd.

GROUP 1—Continued.

SCENEDESMUS QUADRICAUDA (Turp.) Breb.

Date.	One part copper sulphate to water, parts—						Check.
	100,000	200,000	300,000	400,000	500,000	1,000,000	
September 14-18	d	d	vfa	$\frac{1}{2}$d	g	g	g
December 7-12	d	vfa	vfa	$\frac{1}{4}$d	g	g	g
January 11-15	vfa	vfa	vfa	$\frac{1}{4}$d	g	g	g

EUGLENA VIRIDIS Ehrb.

Date.	One part copper sulphate to water, parts—						Check.
	100,000	200,000	300,000	400,000	450,000	500,000	
September 21-25	d	vfa	vfa	$\frac{2}{3}$d	$\frac{1}{2}$d	g	g
October 26-30	vfa	vfa	vfa	$\frac{3}{4}$d	$\frac{1}{2}$d	g	g
December 31-January 2	vfa	vfa	vfa	$\frac{1}{2}$d	$\frac{1}{2}$d	g	g

SPIROGYRA STRICTA (E. Bot.) Wille.

Date.	One part copper sulphate to water, parts—						Check.
	50,000	75,000	100,000	200,000	500,000	1,000,000	
December 26-30	d	vfa	$\frac{3}{4}$d	g	g	g	g

GROUP 2.

CONFERVA BOMBYCINUM Ag.

Date.	One part copper sulphate to water, parts—						Check.
	50,000	100,000	300,000	500,000	1,000,000	2,000,000	
October 1-4	d	d	d	d	d	g	g
October 8-11	d	d	d	vfa	vfa	g	g
October 13-17	d	d	d	vfa	vfa	g	g

CLOSTERIUM MONILIFERUM (Bory) Ehrb.

Date.	One part copper sulphate to water, parts—					Check.
	25,000	100,000	500,000	1,000,000	2,000,000	
December 14-18	d 12hrs	d 24hrs	d	d	$\frac{1}{2}$d	g

SYNURA UVELLA Ehrb.

Date.	One part copper sulphate to water, parts—						Check.
	250,000	500,000	666,666	750,000	1,000,000	2,500,000	
March 14	d 5-25min	d 15-30min	d 15-45min	d 15-60min	d 28-60min	g at 1hr	g at 1hr
March 18	d 5-25min	d 15-30min	d 15-45min	d 15-60min	d 28-60min	g at 1hr	g at 1hr

Effect of various concentrations of copper sulphate upon different forms of algæ—Cont'd.

GROUP 2—Continued.

ANABÆNA CIRCINALIS Raben.

Date.	One part copper sulphate to water, parts—						Check.
	50,000	100,000	500,000	1,000,000	3,000,000	5,000,000	
December 26–29	d	d	d	d	$\frac{1}{2}$d	vfd	g
January 4–7	d	d	d	d	$\frac{1}{4}$d	vfd	g

ANABÆNA FLOS-AQUÆ Breb.

Date.	One part copper sulphate to water, parts—						Check.
	50,000	100,000	500,000	1,000,000	3,000,000	5,000,000	
July 12–14	d 12hrs	d 24hrs	d 24hrs	d 36hrs	d 72hrs	$\frac{1}{2}$d	g
August 27–29	d 12hrs	d 24hrs	d 24hrs	d 36hrs	d 72hrs	$\frac{1}{2}$d	g

GROUP 3.

UROGLENA AMERICANA Calk.

Date.	One part copper sulphate to water, parts—				Check.
	1,000,000	2,500,000	5,000,000	10,000,000	
March 19, 1903	d 3–5min	d 16hrs	vfa 16hrs	vfa 16hrs	g

The foregoing tables clearly demonstrate the effectiveness of copper sulphate as an agent for the destruction of algæ, and as the cost for an amount of this salt necessary to make the strongest solution required will not exceed from 50 to 60 cents per million gallons, but one condition remains to be satisfied—that it shall be absolutely harmless to man, domestic animals, and fish under the conditions used.

In general, animal life is less susceptible to injury by copper than is plant life, though most of the higher plants, some of the fungi, and, as the preceding tables show, certain algæ will live in concentrations of copper sulphate that would be fatal in a few hours to fish and frogs. The critical concentration for game fish is higher than that for such fish as carp and catfish. Black bass in good condition have endured concentrations of 1 to 50,000 for many weeks with no apparent discomfort, while 1 to 100,000 was sufficient to kill German and mirror carp in a few hours, and 1 to 500,000 killed the most susceptible in a few days. Mud catfish are affected at practically the same concentration; goldfish at slightly greater, while yellow perch are perhaps less susceptible than goldfish. This agrees with the results of Perry and Adams,[a] who state that minnows and goldfish live indefinitely in a 1 to 200,000 solution.

[a] Perry & Adams, 4th Rept. River Polut. Conn., 2: 377–391.

The effects of copper upon the higher animals have been studied by a large number of investigators, and the following results may be appropriately cited:

Metallic copper and its oxides, mixed with sugar, albuminoids, and fats, had no noticeable effect upon dogs; even 8 grams of fine powder (4 grams each of copper monoxide and dioxide) caused only a slight sickness. Verdigris in small amounts produced none of the violent results it is supposed to cause in man. Soluble salts of copper can be given in quantities up to 1 gram daily, but more than this has a fatal effect.[a]

Dogs that had eaten half a gram of copper acetate per day for 24 days suffered but slightly; one dog was unaffected by doses as high as 5 grams at a time.[b] Similar results were obtained by Du Moulin,[c] who gave dogs and rabbits as much as 3 to 5 grams, causing sickness but in no case death, and Hippolyte Kuborn[d] states that a dog can take 4 grams of copper sulphate with but slight effect.

Ellenberger and Hofmeister[e] experimented with sheep, giving them from 18 to 182½ grams of copper in quantities sometimes as large as 2 grams per day, with fatal results. Tschirsch[f] deduced from this that the nontoxicity of weak solutions of copper does not hold for ruminants, but this seems hardly warranted. Two grams per day can scarcely be considered a small amount, yet one sheep lived 53 days and the other 128.

Ever since copper compounds have come into general use as fungicides, the question as to their effect upon the human system has received more or less attention.[g] At times there have been vague and misleading statements in the public press, calculated to alarm those who are in the habit of using vegetables and fruits which have been subjected to treatment with Bordeaux mixture. The popular belief seems to be that copper is a poison, but it is found upon examination that the very best authorities are by no means agreed upon this point. It is true that after the question had been discussed for seven months before the Belgian Royal Academy of Medicine, in 1885, it was finally decided that copper compounds in foods were harmful, but it should be remembered that in the whole discussion, where every effort was made by one side to show that copper was an actual poison, not a

[a] Burcq & Ducom, Journal de Pharmacie et Chimie, **25**: 546, 1877.

[b] Galippe, Journal de Pharmacie et Chimie, **23**: 298.

[c] Du Moulin, Journal de Pharmacie et Chimie, **5**: 189.

[d] Hippolyte Kuborn, Congrès Internationale d'Hygiène, **2**: 216, 1878.

[e] Ellenberger and Hofmeister, Archiv für wissench. u. prakt. Thierheilkunde, **9**: 325, 1883.

[f] Tschirsch, Das Kupfer vom Standpunkte der gerichtlichen Chemie, Toxicologie und Hygiene, Stuttgart, 1893.

[g] Spraying Fruits for Insect Pests and Fungous Diseases, with a Special Consideration of the Subject in Its Relation to the Public Health. U. S. Department of Agriculture, Farmers' Bulletin No. 7, 1892. See also Bull. No. 6, Div. Veg. Path., U. S. Dept. Agric.

single instance was given of injury to health resulting from the daily absorption of a small quantity of copper. On the other hand, many instances were cited where foods containing copper in considerable amounts were used without producing any harmful effect whatever. It should be noted also that the law prohibiting the use of copper in regreening fruits was repealed by the French authorities after the discussion before the Belgian Academy.

According to Thiemann-Gartner,[a] chronic copper poisoning has never been proved. The supposed copper colic was discussed by Burcq[b] before the Congrès Internationale d'Hygiène in 1878, and declared by him to have no existence; he even went so far as to assert an immunity against cholera for the workers in copper during various epidemics at Paris, Toulon, Marseilles, and elsewhere, but this statement he afterwards modified with reference to the epidemic of 1832. The good health of copper workers is also noted by Houlès and Pietra-Santa,[c] though they do not claim for them immunity from typhoid and cholera. Gautier[d] states that persons working in dye factories, where the hands, faces, and even hair were colored green by copper, were physically unaffected, which is true also of copper turners, who remain apparently in the best of health although constantly in an atmosphere highly charged with copper dust.

A considerable number of experiments have been made to determine the effect of copper upon man when taken into the intestinal tract. For fourteen months Galippe[e] and his family used food cooked and cooled in copper vessels, the amount of copper present in the food being sufficient to be easily determined. Kobert's experiments[f] show that a 60-kg. man can take 1 gram of copper per day with perfect safety. From his own results Lehmann[g] considers that copper to the amount of 0.1 gram in vegetables may produce bad taste, nausea, possibly colic and diarrhea, but nothing more serious. He has himself found peas containing as much as 630 mg. of copper per kilogram not distasteful, and 200 mg. consumed at a single meal was without effect. A very careful and thorough series of tests have shown that some individuals, at least, can take copper even to the amount of 400 to 500 mg. daily for weeks without detriment to their health.

Tschirsch[h] finds that 0.01 to 0.02 of copper (0.039 to 0.078 of copper sulphate) in dilute form have no effect; 0.05 to 0.2 causes only vomiting and diarrhea.

[a] Thiemann-Gartner, Handbuch und Beurtheilung der Untersuchung der Wasser, Braunschweig, 1895.

[b] Burcq, Congrès Internationale d'Hygiène, **1**: 529, 1878.

[c] Houlès and Pietra-Santa, Journal de Pharmacie et Chimie, 5th Ser., **9**: 303.

[d] Gautier, Le Cuivre et le Plomb, Paris. 1883.

[e] Galippe, Compt. Rend., **84**: 718.

[f] Kobert, Lehrbuch der Intoxicationen. (Original not consulted.)

[g] Lehmann, Münch. Med. Wochensch., **38**: 603.

[h] Tschirsch, l. c.

The process of regreening legumes is described by Bouchardat and Gautier,[a] showing the amount of copper thus introduced into the vegetables to be too small to produce any injurious effect. The maximum amount of this metal in regreened peas as given by Gautier[b] is 125 mg. per kilogram, in connection with which he notes that Chatin and Personne have given it as 270 mg. According to Gautier, the amount of copper ordinarily consumed in a full meal is 95 mg.

Lafar[c] attributes the green color of Lodisan and Parmesan cheese to the presence of copper, giving the maximum amount for Lodisan cheese as 215 mg. per kilogram. Chocolate[d] contains 0.005 to 0.125 gram per kilogram, cafe bourbon[e] 8 mg. per kilogram, and beef 1 mg. per kilogram. There is 0.01 gram of copper sulphate in 1½ pounds of bread,[f] 0.1 gram of copper oxide has been found in 1 kilogram of preserves, and similar amounts are normally present in a large number of commodities used for food.

Medicinal uses of copper compounds are cited by Du Moulin.[g] He has prescribed 12 to 15 cg. for scrofulous children, for cases of ophthalmia, etc., and found no ill effects. Copper sulphate in doses of 40 to 50 cg. for four or five days has proved beneficial to children with diphtheria.

Summarizing from a large number of experiments, Bernatzik[h] concludes as follows: After entering the stomach only small quantities of copper are absorbed by the blood, and toxic action occurs only when the necessary amount can accumulate in the circulation. Silver, copper, and zinc have almost the same medicinal properties, the difference being of degree rather than kind. They differ markedly from other heavy metals, having no harmful effects upon the tissues, and producing no fatal functional injuries; hence they are not poisons in the same sense as are lead, mercury, arsenic, antimony, and phosphorus. Moreover, in the case of copper, after suspension of the dose the injured functions return to the normal.

It is evident that there is still a considerable difference of opinion among eminent authorities as to the exact amount of copper which may be injurious, but as a very conservative limit we may accept 0.02 gram as the amount that may with safety be absorbed daily. According to Merck's Index, the National Dispensatory, and the United States Dispensatory, the dose of copper sulphate for tonic and astrin-

[a] Bouchardat and Gautier, Congres Internationale d'Hygiene, **5**: 486.

[b] Gautier, l. c.

[c] Lafar, Technical Mycology, 159.

[d] Duclaux, Bull. de la Soc. Chim. de Paris, **16**: 35.

[e] Sargeau, Jour. de Pharm., **18**: 219, 654; **16**: 507.

[f] Tschirsch, l. c.

[g] Du Moulin, Journal de Pharmacie et Chimie, **13**: 189.

[h] Bernatzik, Encyclop. d. ges. Medicin., **11**: 429; Encyclop. d. ges. Heilkunde, **11**: 429.

gent purposes is one-fourth grain, or 0.016 gram; as an emetic, a dose of five grains, or 0.33 gram. Thus it is seen that even if the maximum concentration of copper sulphate necessary to destroy algæ in reservoirs were maintained indefinitely, the total absorption from daily use would be very far below an amount that could produce the least unpleasant effect. Taking a dilution of one to one million, which in all cases would be sufficient to prevent the growth of a polluting algal form, it would be necessary to drink something over twenty quarts of water a day before an amount which is universally recognized as harmless would be introduced into the system, while more than fifty quarts would have to be consumed before there would be danger of producing an unpleasant or undesirable effect. As will be seen from the preceding tables the use of copper sulphate at this maximum strength of one to one million would need to be resorted to only in extreme cases, and for a very short length of time, for, the reservoir once entirely free from the organisms, a very much weaker solution would be sufficient should any further application be necessary.

Perhaps the strongest argument in favor of using a chemical treatment of this kind is that even though enough copper should be added to a reservoir to make a one-millionth solution, nothing like this amount would appear in the water distributed. A very large percentage of the copper is combined with the algæ and precipitated in other ways, so that practically none would remain in solution after the first few hours.[a] Samples of water taken from a reservoir treated with sufficient copper sulphate to make a solution of one to one million, failed to show any reaction for copper after twenty-four hours, although all the algæ were killed. It is believed that the process used of evaporating down the original quantity and testing by the delicate potassium ferro-cyanide method would certainly have detected copper had it been present in the proportion of one to fifty million. Other tests were made by different chemists, but always with negative results.

In addition to the use of copper sulphate in reservoirs containing water to be used for domestic purposes, there are possibilities of its application in treating irrigation reservoirs, small pleasure lakes, fish ponds, oyster beds, etc. Here it may often be desirable to exceed the strength of solution that would represent the maximum required in a municipal water supply. This would be done not only to kill all the algæ, but to destroy or drive away reptiles and other pests, leaving the water perfectly clear and clean. The use of some such method for the destruction of mosquito larvæ also seems worthy of attention. The mere removal of the great mass of algal growths in stagnant pools undoubtedly reduces the number of larvæ by destroying this source

[a] Adsorption, according to True and Ogilvie (Science, N. S., **19**: 421), would materially reduce the quantity of copper in solution. See also Bull. No. 9, Veg. Phys. and Path., U. S. Dept. Agric.

of their food and depriving them of protection from fish and other enemies. This is probably the explanation of the reported[a] decrease in the number of mosquito larvæ after spraying a lily pond with Bordeaux mixture, although it is possible that the strength of the solution used may have been partly responsible for their death. It is believed that it will not be impracticable to use the amounts of copper sulphate necessary to actually destroy such larvæ. Certainly this method if effective offers considerable advantages over any now in use, and it should be thoroughly tested. Cooperative experiments are now under way with the Bureau of Entomology to determine the strength of solution necessary to kill larvæ of different species and ages under various conditions.

METHOD OF APPLYING THE COPPER SULPHATE.

The method of introducing the copper sulphate into a water supply is extremely simple. Though any plan will suffice which distributes the copper thoroughly, the one recommended and used by the Department of Agriculture is as follows: Place the required number of pounds of copper sulphate in a coarse bag—gunny-sack or some equally loose mesh—and, attaching this to the stern of a rowboat near the surface of the water, row slowly back and forth over the reservoir, on each trip keeping the boat within 10 to 20 feet of the previous path. In this manner about 100 pounds of copper sulphate can be distributed in one hour. By increasing the number of boats, and, in the case of very deep reservoirs, hanging two or three bags to each boat, the treatment of even a large reservoir may be accomplished in from four to six hours. It is necessary, of course, to reduce as much as possible the time required for applying the copper, so that for immense supplies with a capacity of several billion gallons it would probably be desirable to use a launch, carrying long projecting spars to which could be attached bags each containing several hundred pounds of copper sulphate.

In waters that have a comparatively high percentage of organic acid it is sometimes advisable to add a sufficient amount of lime or some alkali hydrate to precipitate the copper. The necessity for this will never occur in a limestone region, as in this case there will always be enough calcium hydrate or carbonate to cause the desired precipitation. The precipitation of copper does not mean the destruction of its toxicity, for experiments conducted in this laboratory have confirmed Rumm's[b] results that the insoluble salts of copper, such as the hydrate, carbonate, and phosphate, are toxic only if they are in contact with the cell, but are highly toxic in that case. In this connection it should be mentioned that Hedrick[a] has described a method for con-

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[a] Hedrick, Gardening, **11**: 295. [b] Rumm, l. c.

trolling the growth of algal scum in lily ponds by the use of Bordeaux mixture which seems to have been temporarily effective. However, the impracticability of using such a mixture is apparent for the destruction of microscopic algæ distributed through a reservoir or a lake containing millions of gallons.

PRACTICAL TESTS OF THE METHOD.

WATER-CRESS BEDS.

The first practical test of the treatment of water for the purpose of killing out extensive growths of algæ was made in the fall of 1901 near Ben, Va., in connection with the cultivation of water cress for market. Water cress is grown there, as well as in other parts of the country, in large quantities during the winter, it being a valuable crop at that season of the year. The cress is confined in beds made by constructing dams across a small stream, which maintains a water level not too high for the growth of the plants and yet permits flooding when there is danger of a freeze. In the locality where the experiments were carried on the water was obtained from a thermal spring with a temperature the year around of about 70° F. Such a temperature was particularly favorable to the development of *Spirogyra* and similar filamentous algæ, so that when the cress was freshly cut they frequently increased to such an extent as to completely smother out a large part of the young and tender plants. The only known remedy under such conditions was to rake out the water cress and algæ and reset the entire bed. This was an expensive method, however, besides being successful only about half the time. Consequently, it was very desirable to devise some means of preventing the growth of the algæ without injuring the water cress, and the treatment by means of copper suggested itself. At first a strong solution of copper sulphate was used, spraying it on the algal covered surface of the beds, but this only destroyed the few filaments with which the copper came in contact, the large mass of algæ being practically unaffected. The method of applying the copper by means of dissolving it directly in the beds was next tried, and the success of the treatment was almost immediately evident. In this case the amount of copper added was about equal to a strength of 1 to 50,000,000 parts of water, but it is probable that by the time it reached most of the *Spirogyra* it was considerably weakened, as it was impossible to prevent a slight current of fresh water from passing through the beds at all times.

The success of the copper treatment for eradicating algæ from cress beds has been thoroughly demonstrated, and there is no reason why growers should have trouble from this cause in the future. The strength of the solution used for killing the algæ is so very much weaker than that which might affect the cress that there is no possible danger of

injuring the latter if the solution is used by anyone capable of observing ordinary care. The question of how long a treatment is effective must, of course, depend upon conditions, but it is believed that the application of the proper amount of copper once or twice a year will in most cases be sufficient to keep down any algal pest. The manager of the Virginia Cress Company writes, under date of April 12, 1904:

> The "moss" has given me no trouble at all this winter. In fact I have for six months only had to resort to the copper sulphate once. * * * All the conditions were favorable last fall and early winter for a riot of "moss," but it did not appear at all until just a few days ago, and then yielded to treatment much more readily than it did when I first began to use the copper.

WATER RESERVOIRS.

The successful elimination of algæ from the cress beds of the South, under conditions which were particularly favorable to the growth of these pests, made it desirable that experiments be inaugurated calculated to demonstrate the possibility of ridding water reservoirs of the disagreeable odors and tastes caused by similar organisms. While it was realized that the popular prejudice against any chemical treatment of drinking water was strong, it was believed that the very weak solution, together with the very rapid disappearance of the salt added, would not render it a prohibitive method when applied under the direction of the proper authorities. It was also found that consumers of a water which possessed a disgusting odor and taste were not so prejudiced against the use of even a chemical method of extermination, provided it could be proved that no bodily harm would result.

In the spring of 1903 there was brought to the notice of the Department the supply of a water company in Kentucky, which promised to furnish a most satisfactory test. Ever since the construction of their reservoir it had given off an unpleasant odor. For the first two seasons this was supposed to be due to decaying vegetation, but later years demonstrated the well-known "pigpen" odor due to algæ, and this increased from year to year until it was almost unbearable.

In July, 1903, when the trial was begun, the microscopical examination demonstrated an average of—

Anabæna	per cc..	7,400
Clathrocystis	do....	1,100
Eudorina	do....	200

There were about 25,000,000 gallons of water in the reservoir at the time of the experiment, and on account of the great number of blue-green algæ present it was decided to apply the copper at a strength of 1 to 4,000,000. About 50 pounds of copper sulphate was accordingly placed in a coarse sack and this, attached to a boat, was dragged over the surface of the reservoir, giving especial attention to the region which seemed to contain the greatest number of *Anabæna* filaments.

The decrease in the number of organisms as the result of this treatment during the next twenty-four hours was very decided. In two days the surface was clear and the water had lost its blue-green color, becoming brown, due to the dead organisms held in suspension. There was a slight increase in odor during the first two days after treatment, but this was followed by a gradual subsidence until it had entirely disappeared, not to appear again that season. The following list of counts made from surface examinations at one station illustrates what went on throughout the reservoir, and shows the almost immediate effect of a 1 to 4,000,000 solution of copper sulphate upon the number of filaments of *Anabæna flos-aquæ*. The treatment was made July 9.

	Filaments per cubic centimeter.
July 6	3,400
July 10	54
July 11	8
July 13	0
July 15	0
July 20	0

It remains to be seen what the condition will be during the coming summer, but it is believed it can never be any worse than at the time of treatment, and it is reasonable to suppose that there will be considerably fewer organisms this year than last. Even though an annual treatment of the reservoir prove necessary, involving a cost of from $25 to $50, the already great improvement in the quality of the water will certainly make it justifiable.

Other experiments of a similar character were carried on in different parts of the country with reservoirs of a capacity of from 10,000,000 to 600,000,000 gallons. While the results were all favorable, it is deemed best not to publish any detailed account until the effect of the treatment can be followed through another season. The summer of 1903 was cold and wet, and in some cases the decrease in the number of organisms may have been due to these factors. However, the several instances of the very sudden and rapid disappearance of forms which were present in tremendous quantity, without any reappearance, indicated that the treatment was most effective. Those in charge of these water supplies reported that they were well satisfied with the result.

EFFECT OF COPPER UPON PATHOGENIC BACTERIA.

TYPHOID.

The value of copper sulphate as an agent for the destruction of algæ polluting reservoirs suggests its use in cases where the organism is pathogenic. Since this salt is fatal to the algal growths, it seemed

probable that it would also destroy bacteria, and that cholera germs and typhoid germs might succumb to its action.

The sterilization of public water supplies by chemical means has so far seemed an impossibility. Nearly every known substance has been tested, but the high concentrations required to produce the desired effect, the extreme toxicity of the agents, their cost, or the difficulty of application, have eliminated all but copper sulphate as a possibility for the present purpose. According to Semmer and Krajewski,[a] a 1 to 160 solution of this salt will inhibit action in infected blood, and septic bacteria can be destroyed with a 10 per cent solution. Bolton[b] says that 1 to 500 is toxic, but 1 to 1,000 permits the growth of cholera; 1 to 200 and 1 to 500, respectively, produce the same results with typhoid, and some of the spore-bearing forms are unaffected at 2 per cent. Green[c] gives 2½ per cent as the amount necessary to kill typhoid in two to twenty-four hours, and finds cholera only slightly less sensitive. Israel and Klingman,[d] however, find that almost infinitesimal amounts of copper in colloidal solution are fatal to typhoid, cholera, and *Bacillus coli.* There is considerable literature upon the use of copper sulphate as a disinfectant for clothing, bedding, cesspools, etc., but it is not necessary to review it at this place. Sternberg[e] found that its germicide power was decidedly superior to the corresponding salt of iron and zinc, and demonstrated that it destroyed micrococci from the pus of an acute abscess in the proportion of 1 to 200. He says, "This agent (cupric sulphate), then, is a valuable germicide and may be safely recommended for the disinfection of material not containing spores."

The high percentage of copper sulphate given by most of these authorities seems to preclude the idea of its practical use for the purpose desired. It should be remembered, however, that these investigators were working for a very different end, namely, to find concentrations destructive to bacteria in the presence of large quantities of albuminoid and fatty matter. Experiments conducted under similar circumstances have confirmed the above results, but the conditions obtaining in public water supplies are widely different. Here the amount of albuminoid matter is so small that the death point of the typhoid or cholera organism is lowered tremendously and very dilute solutions of copper are shown to be toxic. The tabulated results on the succeeding pages demonstrate this fact.

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[a] Semmer and Krajewski, Arch. f. exp. Path. u. Pharmakol., **14**: 139.

[b] Bolton, Rep. of Com. on Disinfectants, Am. Pub. Health Assn., 1888, p. 153.

[c] Green, Zeit. für Hyg., **13**: 495.

[d] Israel and Klingman, Virchon's Archiv., **147**: 293.

[e] Sternberg, Rep. Com. Disinfection, Am. Pub. Health Assn., 1888, p. 38. See also Infection and Immunity, New York and London, 1903.

Effect of copper sulphate upon Bacillus typhi at different temperatures. [a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' incubation; — indicates no growth.]

Duration of exposure to action of copper sulphate.	Temperature.	Check.	1 part copper sulphate to 100,000 parts of water.	1 part copper sulphate to 200,000 parts of water.	1 part copper sulphate to 500,000 parts of water.
	° *C.*				
2 hours	38	+	—	+	+
	28	+	+	+	+
	23.5	+	+	+	+
	14	+	+	+	+
	4	+	+	+	+
4 hours	38	+	—	+	+
	28	+	—	+	+
	23.5	+	(?)	+	+
	14	+	+	+	+
	4	+	+	+	+
6 hours	38	+	—	—	+
	28	+	—	+	+
	23.5	+	—	+	+
	14	+	+	+	+
	4	+	+	+	+
12 hours	38	+	—	—	+
	28	+	—	+	+
	23.5	+	—	+	+
	14	+	—	+	+
	4	+	(?)	+	+

[a] Experiment conducted in test tubes, each containing 5 cc. of sterilized water, portions of which had been previously treated with the desired amounts of copper sulphate. All tubes inoculated with a 3 mm. loop of a 24-hour culture of *B. typhi.*

Effect of copper sulphate upon Bacillus typhi cultures of various ages. [a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' incubation; — indicates no growth.]

Duration of exposure to action of solution of 1 part copper sulphate to 100,000 parts of water.	Culture 36 hours old.	Culture 24 hours old.	Culture 18 hours old.	Culture 12 hours old.	Culture 6 hours old.	Culture 3 hours old.
3 hours	+	+	+	+	—	—
6 hours	(?)	—	—	—	—	—
9 hours	—	—	—	—	—	—

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, portions of which had been previously treated with the desired amount of copper sulphate. All tubes inoculated with a 3 mm. loop of a culture of *B. typhi* of the proper age.

Effect of copper sulphate on Bacillus typhi at different temperatures. [a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper sulphate.	Temperature.	Check.	One part copper sulphate to 100,000 parts of water.	One part copper sulphate to 200,000 parts of water.	One part copper sulphate to 500,000 parts of water.
	° *C.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
2 hours	5	720	315	1,440	894
2 hours	38	1,260	0	312	917
5 hours	5	155	115	495	278
5 hours	38	37	0	9	21

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, portions of which had been previously treated with the proper amounts of copper sulphate. All tubes inoculated with a 3 mm. loop of an 18-hour culture of *B. typhi.*

Effect of copper sulphate upon Bacillus typhi at room temperature.[a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper sulphate.	Check.	One part copper sulphate to—				
		100,000 parts water.	200,000 parts water.	500,000 parts water.	1,000,000 parts water.	5,000,000 parts water.
		Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
½ hour	1,650	5,481	2,376	2,754	2,646	3,645
1 hour	1,836	918	2,106	2,403	1,377	1,755
1½ hours	1,566	1,026	1,242	1,323	2,673	2,808
2 hours	1,485	864	1,296	2,835	2,430	3,024
2½ hours	999	243	1,620	1,485	2,727	2,106
3 hours	1,134	180	1,161	1,620	1,782	756
3½ hours	1,080	156	783	918	2,079	1,242
4 hours	783	108	972	1,998	1,836	1,458
8 hours	270	0	72	405	324	459
12 hours	297	0	14	42	243	405

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, portions of which had been previously treated with the desired amounts of copper sulphate. All tubes inoculated with a 3 mm. loop of an 18-hour culture of *B. typhi*.

Effect of copper sulphate upon Bacillus typhi at room temperature.[a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper sulphate.	No. 1. Check.			No. 2. One part copper sulphate to 200,000 parts water.			No. 3. One part copper sulphate to 100,000 parts water.			No. 4. One part copper sulphate to 50,000 parts water.			No. 5. One part copper sulphate to 100,000 parts water.		
	Colonies.			Colonies.			Colonies.			Colonies.			Colonies.		
	Bacillus typhi.	Molds.	Saprophytic bacteria.	Bacillus typhi.	Molds.	Saprophytic bacteria.	Bacillus typhi.	Molds.	Saprophytic bacteria.	Bacillus typhi.	Molds.	Saprophytic bacteria.	Bacillus typhi.	Molds.	Saprophytic bacteria.
0 hour	144	4	5	108	2	7	3	1	4	3,672	0	0	234	0	5
	792	2	4	90	1	4	198	1	5	5,742	1	1	306	0	0
3 hours	14,634	2	7	11	0	5	72	3	4	0	0	0	6	0	0
	16,212	0	0	126	0	2	6	0	0	4	0	1	4	1	0
4 hours	954	0	2	0	0	1	0	0	0	0	0	0	0	2	0
	558	3	31	0	0	0	0	0	0	0	0	3	0	0	0
6 hours	24,300	2	8	0	1	1	0	0	1	0	0	0	0	0	0
	19,400	0	0	0	1	5	0	0	0	0	0	3	0	1	1
8 hours	20,484	0	0	0	0	2	0	0	1	0	0	0	0	0	0
	19,674	0	0	0	0	0	0	0	3	0	0	2	0	1	0
12 hours	6,156	0	33	0	0	0	0	0	0	0	0	0	0	0	0
	21,600	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Experiment conducted in 12-liter aquaria. No. 1 was untreated; copper sulphate was added to Nos. 2, 3, 4, and 5. Three cubic centimeters of a mixture of cultures of *B. typhi* were added to each jar 18 hours before treating. All small nonliquifying colonies counted as typhoid.

Effect of copper sulphate upon Bacillus typhi at low temperature.[a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper sulphate.	Temperature.	Check.	One part copper to 100,000 parts water.
	° C.	*Colonies.*	*Colonies.*
3 hours	5	2,187	1,944
6 hours	5	2,646	881
9 hours	5	1,026	702
12 hours	5	351	98
24 hours	5	37	0

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, part of which had been previously treated with the desired amount of copper sulphate. All tubes inoculated with a 3 mm. loop of a culture of *B. typhi* of the proper age.

Effect of copper sulphate upon Bacillus coli cultures of various ages.[a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' incubation; – indicates no growth.]

Duration of exposure to action of solution of 1 part copper sulphate to 100,000 parts water.	Culture 36 hours old.	Culture 24 hours old.	Culture 18 hours old.	Culture 12 hours old.	Culture 6 hours old.	Culture 3 hours old.
3 hours	+	+	+	+	+	–
6 hours	–	–	+	–	–	–
9 hours	–	–	+	–	–	–

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, part of which had been previously treated with the desired amount of copper sulphate. All tubes inoculated with a 3 mm. loop of a culture of *B. coli* of the proper age.

Effect of copper sulphate upon Bacillus coli at different temperatures.[a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' incubation; – indicates no growth.]

Duration of exposure to action of copper sulphate.	Temperature.	Check.	One part copper sulphate to— 100,000 parts water.	200,000 parts water.	500,000 parts water.
	° C.				
2 hours	38	+	+	+	+
	28	+	+	+	+
	23.5	+	+	+	+
	14	+	+	+	+
	4	+	+	+	+
4 hours	38	+	–	+	+
	28	+	+	+	+
	23.5	+	+	+	+
	14	+	+	+	+
	4	+	+	+	+
6 hours	38	+	–	+	+
	28	+	+	+	+
	23.5	+	+	+	+
	14	+	+	+	+
	4	+	+	+	+

[a] Experiment conducted in test tubes each containing 5 cc. sterilized water, portions of which had been previously treated with the desired amounts of copper sulphate. All tubes inoculated with a 3-mm. loop of a 24-hour culture of *B. coli*.

Effect of copper sulphate upon Bacillus coli at room temperature. [a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper sulphate.	Check.	1 part copper sulphate to— 100,000 parts of water.	200,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.	5,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
½ hour	3,888	5,697	4,455	8,937	5,490	6,426
1 hour	3,456	2,295	1,755	2,700	3,483	2,160
1½ hours	2,592	2,565	1,755	2,403	1,377	1,873
2 hours	2,079	1,971	3,429	1,890	3,267	3,942
2½ hours	3,969	2,835	2,295	3,456	2,214	2,349
3 hours	2,457	1,701	1,242	3,834	2,106	3,078
3½ hours	1,566	1,404	2,295	1,431	2,025	3,240
4 hours	1,323	675	1,593	2,403	1,674	1,836
8 hours	1,107	96	459	1,026	513	1,728
12 hours	297	5	43	366	513	891

[a] Experiment conducted in test tubes, each containing 5 cc. of sterilized water, portions of which had been previously treated with the desired amounts of copper sulphate. All tubes inoculated with a 3 mm. loop of an 18-hour culture of *B. coli.*

Effect of copper sulphate upon Bacillus coli at low temperature. [a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper sulphate.	Temperature.	Check.	1 part copper to 100,000 parts water.
	° *C.*	*Colonies.*	*Colonies.*
3 hours	5	2,700	2,673
6 hours	5	3,591	1,620
9 hours	5	2,403	1,215
12 hours	5	2,106	1,431

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, part of which had been previously treated with the desired amount of copper sulphate. All tubes inoculated with a 3 mm. loop of a culture of *B. coli* of the proper age.

Effect of copper sulphate upon paracolon cultures of various ages. [a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' incubation; — indicates no growth.]

Duration of exposure to action of solution of 1 part copper sulphate to 100,000 parts of water.	Culture 36 hours old.	Culture 24 hours old.	Culture 18 hours old.	Culture 12 hours old.	Culture 6 hours old.	Culture 3 hours old.
3 hours	+	—	?	—	—	—
6 hours	—	—	—	—	?	—
9 hours	—	—	—	—	—	—

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, part of which had been previously treated with the desired amount of copper sulphate. All tubes inoculated with a 3 mm. loop of a culture of paracolon of the proper age.

These tables show that *Bacillus typhi* is more sensitive to copper sulphate than is *coli*, that the para group are about equally sensitive, and that temperature has a very important bearing on the toxicity of

the copper in solution. At room temperature, which is near the temperature of a reservoir in summer, a dilution of 1 to 100,000 is fatal to *typhi* in three to five hours; at 5° it requires twenty-four hours for complete destruction.

The results obtained were checked in three ways:

(1) Five cubic centimeters of each of the solutions to be tested, made up with filtered hydrant water and check tubes of the same water, were sterilized in test tubes. To each of these was transferred one 3-mm. loop of a bouillon culture of the bacillus. After the proper exposure, a 3-mm. loop of the inoculated water from each tube was transferred to a sterile bouillon tube with a corresponding number. These bouillon tubes were then incubated forty-six hours at 38°, the time and concentration of the agent required to prevent growth being noted.

(2) Instead of transferring to bouillon tubes from the inoculated water, the transfer was made to gelatine tubes, and plates were poured in 10-cm. Petri dishes, thus making it possible to estimate the reduction in the number of bacteria in concentrations not sufficient to prevent growth.

(3) Five 12-liter aquaria, two of which contained a high percentage of organic matter, also a large quantity of algæ and other aquatic plants, were inoculated, each with 3 cubic centimeters of cultures of *Bacillus typhi* of different ages, and allowed to stand eighteen hours, and two poured plates were made from each aquarium, the 3-mm. loop being used in all cases. To these aquaria were then added a 1 per cent solution of copper sulphate in sufficient quantity to produce the desired concentration. After the proper time had elapsed, another series of plates was made, this being repeated every two hours for a period of twelve hours.

The tests were made upon four distinct cultures of *Bacillus typhi*, designated respectively Wasserman, Stokes, Say, and Longcope, and except in the case of the aquaria series, upon *Bacillus coli* and some of the para forms. These organisms were obtained from the laboratory of H. K. Mulford & Co.

ASIATIC CHOLERA.

The method of procedure in determining the toxic concentration for *Microspira comma* (*Spirillum choleræ*) was identical to that employed in the case of *Bacillus typhi*. The tables on the next page show that the toxic limits of these two pathogenic organisms are very similar and that *Microspira comma* is slightly more sensitive to copper sulphate than is *Bacillus typhi*. To destroy the cholera germ requires about three hours in a 1 to 100,C00 solution at a temperature above 20°. A longer exposure or a higher concentration is necessary to produce this result at lower temperatures.

Effect of copper sulphate upon Microspira comma at different temperatures. [a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper sulphate.	Temperature.	Check.	One part copper sulphate to— 100,000 parts water.	200,000 parts water.	500,000 parts water.
	° *C.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
2 hours	5	1,866	1,400	566	3,366
	15	2,500	533	1,100	1,000
	26	3,500	3	100	733
	30.5	4,556	7	66	1,433
4 hours	5	1,533	133	13	766
	15	1,033	21	72	95
	26.5	1,033	0	6	11
	30.5	1,466	0	0	12
6 hours	5	2,000	32	9	700
	15	3,033	9	20	84
	26.5	3,600	0	166	533
	30.5	1,066	0	0	90

[a] Experiments conducted in test tubes, each containing 5 cc. of sterilized water, portions of which had been previously treated with the desired amounts of copper sulphate. All tubes inoculated with a 3 mm. loop of a 14-hour culture of *M. comma*.

Effect of copper sulphate upon Microspira comma at different temperatures. [a]

[Determinations made in buillon tubes. + indicates growth after 48 hours' incubation; – indicates no growth.]

Duration of exposure to action of copper sulphate.	Temperature.	Check.	1 part of copper sulphate to— 100,000 parts water.	200,000 parts water.	500,000 parts water.
	° *C.*				
2 hours	17	+	+	+	+
	24.4	+	+	+	+
	30.5	+	–	–	+
4 hours	17	+	+	+	+
	24.4	+	–	+	+
	30.5	+	–	–	+
6 hours	17	+	–	+	+
	24.4	+	–	+	–
	30.5	+	–	–	–

[a] Experiment conducted in test tubes each containing 5 cc. of sterilized water, part of which had been previously treated with the desired amount of copper sulphate. All tubes inoculated with a 3 mm. loop of a 16-hour culture of *M. comma*.

It will be seen that the concentration of copper required is considerably greater than the maximum necessary for the destruction of algæ, and would, of course, be injurious to the aquatic animals normally present in a reservoir if it were allowed to act for any great length of time. Experiments in this laboratory have demonstrated, however, that the time necessary to remove *Bacillus typhi* is from three to four hours in summer, twenty-four hours in the coldest weather, and that under such conditions the solution does not injure fish and frogs or the common aquatic plants such as *Elodea*, *Myriophyllum*, and *Lemna*. To remove the copper at the desired time the method

suggested in the preceding section in the case of acid and soft waters may be employed—that is, precipitate the copper by some soluble hydroxide or carbonate. This somewhat complicates the treatment, as it will be necessary to determine from the character of the water the amount of copper necessary to produce a solution of 1 to 100,000, as well as to estimate how much of the hydroxide or carbonate should be added. That such work be conducted under the constant and direct supervision of competent authorities is even more important than when treating for algal contamination.

COMPARISON OF EFFECT OF OTHER DISINFECTANTS.

A comparison of the effect of copper sulphate with certain other substances commonly used as disinfectants is instructive, and gives some idea of the great toxicity of this metal. Mercuric chloride (corrosive sublimate) is slightly more fatal to typhoid and cholera than copper sulphate acting at a lower temperature and in a shorter length of time. Carbolic acid, one hundred times as strong as the dilution found to be effective for copper sulphate, and acting eight times as long, failed to kill. The same is true of formalin used between fifteen and twenty times the strength of a 1 to 100,000 solution. Using one thousand times the amount of citric acid that would be used of copper sulphate produces death. Thymol is effective in six hours when used in a solution of 1 to 5,000, and naphthalene is five times weaker.

COLLOIDAL SOLUTIONS.

The preceding experiments have dealt with copper in solution as the salt of some acid. The effect upon water of metallic copper surfaces, producing the so-called colloidal solution of copper, deserves especial mention. As Nägeli, Galeotti, and Israel and Klingman have abundantly demonstrated, the slight amounts of copper thus brought into solution are highly toxic to many forms of algæ and bacteria.

The experiments carried on in this laboratory show that it is undoubtedly possible to exterminate *Uroglena* and some forms of *Spirogyra* by suspending in the water copper foil sufficient to give an area of about 1 sq. cm. to each 100 cc. of water. This would not be a practicable method of treating a reservoir, but it suggests the possibility of sheet copper being used as a preventive of pollution. By suspending large sheets of this metal at the intake of a reservoir, it is probable that conditions would be rendered sufficiently antagonistic to algal growth to maintain the sterility of a reservoir after it had once been thoroughly cleansed of polluting forms. It would, of course, be necessary to keep such copper sheets clean in order to prevent a reduction of the toxic action due to the formation of an insoluble or slimy coating on its surface. It is possible that some

electrical method may be perfected for rapidly obtaining a strong colloidal solution, which will furnish a more convenient means of application than that of the crude salt.

In regard to the bacteria causing cholera and typhoid, the importance of the specific toxic effect of colloidal copper is probably much greater than with algæ. The following tables show the proportions of the area of copper to the quantity of water and to the time and the temperature necessary to produce the complete sterilization of water containing these pathogenic germs:

Effect upon Bacillus typhi of exposure to colloidal solution of copper at room temperature.[a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' inoculation; – indicates no growth.]

Duration of exposure to action of copper.	Check.	15 sq. mm. copper foil in 10 cc. of water.	100 sq. mm. copper foil in 10 cc. of water.	225 sq. mm. copper foil in 10 cc. of water.
10 hours	+	+	+	+
16 hours	+	+	+	--
20 hours	+	+	–	–
50 hours	+	+	–	–

[a] Experiment conducted in test tubes containing 10 cc. each of sterilized water. The copper foil was sterilized and added immediately before inoculating the tubes with the usual 3 mm. loop of a 24-hour culture of *B. typhi*. This experiment was duplicated with three separate strains of typhoid with identical results.

Effect upon Bacillus typhi of exposure to colloidal solution of copper at room temperature.[a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper.	Check.	1 sq. cm. copper foil to 5 cc. of water.	4 sq. cm. copper foil to 5 cc. of water.
	Colonies.	*Colonies.*	*Colonies.*
½ hour	1,650	2,241	2,025
1 hour	1,836	1,944	2,349
1½ hours	1,566	1,620	1,188
2 hours	1,485	1,674	1,188
2½ hours	999	675	1,053
3 hours	1,134	972	918
3½ hours	1,080	1,242	621
4 hours	783	837	360
8 hours	270	216	0
12 hours	297	24	0

[a] Experiment conducted in test tubes, each containing 5 cc. of sterilized water. The copper foil was sterilized, and added immediately before inoculating the tubes with the usual 3 mm. loop of a 24-hour culture of *B. typhi*.

Effect upon Bacillus coli of exposure to colloidal solution of copper at room temperature.[a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' inoculation; – indicates no growth.]

Duration of exposure to action of copper.	Check.	15 sq. mm. copper foil in 10 cc. of water.	100 sq. mm. copper foil in 10 cc. of water.	225 sq. mm. copper foil in 10 cc. of water.
10 hours	+	+	+	+
16 hours	+	+	+	–
20 hours	+	+	+	–
50 hours	+	+	+	–

[a] Experiment conducted in test tubes containing 10 cc. each of sterilized water. The copper foil was sterilized and added immediately before inoculating the tubes with the usual 3 mm. loop of a 24-hour culture of *B. coli.*

Effect upon Bacillus coli of exposure to colloidal solution of copper at room temperature.[a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper.	Check.	1 sq. cm. copper foil to 5 cc. of water.	4 sq. cm. copper foil to 5 cc. of water.
	Colonies.	*Colonies.*	*Colonies.*
½ hour	3,888	2,241	3,024
1 hour	3,456	1,971	2,025
1½ hours	2,592	1,512	2,754
2 hours	2,079	1,188	1,846
2½ hours	3,969	1,242	999
3 hours	2,457	1,242	1,593
3½ hours	1,566	1,026	2,727
4 hours	1,323	1,323	810
8 hours	1,107	702	69
12 hours	297	348	0

[a] Experiment conducted in test tubes, each containing 5 cc. of sterilized water. The copper foil was sterilized and added immediately before inoculating the tubes with the usual 3-mm. loop of a 24-hour culture of *B. coli.*

Effect upon paracolon of exposure to collodial solution of copper at room temperature.[a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' inoculation; – indicates no growth.]

Duration of exposure to action of copper.	Check.	15 sq. mm. copper foil in 10 cc. of water.	100 sq. mm. copper foil in 10 cc. of water.	225 sq. mm. copper foil in 10 cc. of water.
5 hours	+	+	+	+
10 hours	+	+	+	–
16 hours	+	+	+	–
20 hours	+	+	–	–
50 hours	+	+	–	–

[a] Experiment conducted in test tubes containing 10 cc. each of sterilized water. The copper foil was sterilized and added immediately before inoculating the tubes with the usual 3 mm. loop of a 24-hour culture of paracolon. This experiment was duplicated upon another form of paracolon with exactly the same results.

Effect upon paratyphoid of exposure to colloidal solution of copper at room temperature. [a]

[Determination made in tubes of bouillon. + indicates growth after 48 hours' inoculation; – indicates no growth.]

Duration of exposure to action of copper.	Check.	15 sq. mm. copper foil in 10 cc. of water.	100 sq. mm. copper foil in 10 cc. of water.	225 sq. mm. copper foil in 10 cc. of water.
10 hours	+	+	+	+
16 hours	+	+	+	–
20 hours	+	+	–	–
50 hours	+	+	–	–

[a] Experiment conducted in test tubes containing 10 cc. each of sterilized water. The copper foil was sterilized and added immediately before inoculating the tubes with the usual 3 mm. loop of a 24-hour culture of paratyphoid.

Effect upon Microspira comma of colloidal solution of copper at various temperatures. [a]

[Determination made in Petri dishes.]

Duration of exposure to action of copper.	Temperature.	Check.	¼ sq. cm. copper foil to 5 cc. water.	2 sq. cm. copper foil to 5 cc. water.
	° *C.*	*Colonies.*	*Colonies.*	*Colonies.*
2 hours	5	1,866	833	2,500
	15	2,500	733	2,433
	26.5	3,500	4,600	333
	30.5	4,556	1,666	533
4 hours	5	1,533	52	29
	15	1,033	633	366
	26.5	1,033	200	0
	30.5	1,466	8	30
6 hours	5	2,000	700	10
	15	3,033	45	17
	26.5	3,600	300	0
	30.5	1,066	4	8

[a] Experiments conducted in test tubes, each containing 5 cc. of sterilized water, portions of which had been previously treated with the desired amounts of copper sulphate. All tubes inoculated with a 3 mm. loop of a 14-hour culture of *M. comma.*

It is evident that the amount of surface exposed in any ordinary copper tank would far exceed the amount demanded for the above results, and it is likewise certain that after standing from 6 to 8 hours at room temperature in a *clean* copper vessel water becomes safe to drink even though it may have contained cholera and typhoid germs. It remains to be seen whether or not the application of these facts to conditions in the Tropics, where cholera is abundant, will be of any value. It would seem that the construction of canteens and other water vessels from copper might serve as an additional safeguard, if not an actual preventive of this disease, and would prove of considerable value where distillation or efficient filtration apparatus is not at hand.

CONCLUSIONS.

It is believed that the foregoing experiments demonstrate the possibility of the use of copper sulphate for the destruction or prevention of growths of algæ in water supplies, and that when used under the direction of a competent authority, it is the only practicable remedy for this trouble capable of universal application which has ever been proposed. It is, of course, probable that with the experience which must come from a wider opportunity for testing this salt, many improvements will be made in the practical application of the treatment to large bodies of water. However, it is hoped that the results already obtained, together with trials now under way, will make it possible to begin using this method within a short time upon a large scale throughout the country.

NECESSITY OF KNOWLEDGE OF ORGANISM AND CONDITION IN RESERVOIR.

It can not be too strongly emphasized, however, that harmless as the method undoubtedly is under proper control, it must always require a certain amount of definite knowledge in regard to the condition of the reservoir before any treatment can be made, even by those thoroughly able to conduct such an experiment. This is regarded as a fortunate requisite, since it will tend to prevent the irresponsible or careless dosing of reservoirs by incompetents, who are occasionally in charge of water supplies.

Before the amount of copper to be added can possibly be known, it is absolutely necessary to ascertain the exact character of the organism causing the trouble. This will make a microscopical examination of the first importance. Also, the sooner such an examination reveals the presence of the polluting form, the more effective will be the treatment. If examinations are made at short intervals during the entire year, it is possible to detect the troublesome forms at their first appearance and by prompt treatment to destroy the algæ before the consumer is aware of any difficulty. The early detection of the algæ will also make a considerable difference in the expense of the treatment, as it may require fifteen or twenty times as much copper to clean a reservoir after the bad odor and taste are evident than it would could the application have been made before the organism began to rapidly multiply. In all cases the use of copper as a preventive rather than a cure is advocated, and this can not be intelligently applied unless the microscopical examinations are thorough and frequent at the time of year the trouble is to be anticipated.

On account of the necessity of determining the nature of the organism and the time of its appearance as nearly as possible, it will become as imperative for water companies to employ some one competent to

make these examinations as it now is to have a chemist or bacteriologist. In fact, in regions where the difficulty from algæ is great, the microscopical examination must take precedence of everything else as a means of keeping the water palatable and satisfactory to the consumer.

In addition to the character of the organisms and the earliest possible determination of their appearance, it has already been pointed out that the chemical constitution, the temperature, and other special conditions of the water are factors in determining the line of treatment. No specific instructions are given in this bulletin for the amount of copper sulphate which is to be used for each species of algæ which is known to affect water supplies, because it is impossible to make a definite statement without a knowledge of the conditions already mentioned. *Each reservoir must be regarded as an individual case, requiring special knowledge and a particular prescription.* It is believed that the public water supplies of this country are worthy of such special care, and it would be a matter of regret if the method proposed here should ever be regarded as a universal panacea to be used by everyone, regardless of the organism to be eradicated and the condition of the water.

APPLICATION OF METHOD FOR DESTRUCTION OF PATHOGENIC BACTERIA NOT DESIGNED TO REPLACE EFFICIENT MEANS OF FILTRATION ALREADY IN USE.

The use of copper sulphate in clearing polluted reservoirs of pathogenic bacteria, such as typhoid and cholera, is regarded as incidental to the main purpose of the investigation. There already exists a most efficient means of preventing the appearance of these organisms in water supplies, and under no circumstances can it be considered that the method as described is expected to replace or supersede slow sand or any other efficient filtration. There are conditions, however, which sometimes make it desirable to thoroughly sterilize a reservoir, and under those circumstances the use of copper sulphate is believed to offer a new and adequate way of dealing with the difficulty. Experience has demonstrated the impossibility of compelling consumers of what may be an infected water to boil it, or observe other precautionary measures, and the absence of proper filtration plants in a very great number of cities and towns in this country makes it necessary that some efficient method for destroying disease germs in water be employed until the danger from pollution be past. Up to this time no satisfactory and yet harmless method has been known that would become effective in the course of a very few hours and the cost of which was in the reach of every community. It is believed that the results of the experiments upon typhoid and cholera germs described in this bulletin indicate that it will be possible under competent direction to employ copper sulphate with perfect safety in any municipal water

reservoir which may have become infected with some nonspore-forming disease germ. Its application to barnyard tanks and pools as a preventive of hog cholera may also prove to be of value. Since the selective toxicity of this salt renders it fatal to pathogenic forms peculiar to water, while the common saprophytic or beneficial bacteria are unaffected, the method is particularly well adapted for this purpose.

MEDICINAL USE.

While it is not within the province of this bulletin to discuss or recommend any line of medical treatment, reference should be made to the fact that certain eminent practitioners, after reviewing the results here published, are of the opinion that the use of copper in cases of typhoid fever and related diseases should be more thoroughly investigated than it has been heretofore. It was the testimony of several that other intestinal troubles, more recently presumed to be due to the presence of certain disease germs in drinking water and milk, had responded most favorably to copper in one form or another.

CONDITIONS UNDER WHICH THE DEPARTMENT OF AGRICULTURE CAN FURNISH INFORMATION AND ASSISTANCE IN APPLYING THIS METHOD.

The problem of destroying or preventing the growth of algæ by the method devised in the laboratory of plant physiology in water reservoirs, lakes, ponds, water-cress beds, and wherever these plants have become a pest, is one which distinctly comes within the province of the Department of Agriculture. Definite instructions as to the treatment to be followed will at all times be furnished to the proper authorities who may desire assistance, and in so far as the limited facilities of the laboratory permit, determination will be made of the organisms causing the trouble. It is earnestly hoped that no tests of the method described here will be made without first consulting with the Department. Those most intimately connected with this work are constantly gaining information and experience, and this may prove of considerable value, besides a saving of expense, to those who have occasion to exterminate algal pests.

The treatment of water supplies for the destruction of pathogenic bacteria, or any application of the copper-sulphate method which has to do with public health, is not contemplated or indeed possible by this Department. The requests of private individuals or unauthorized bodies for information or assistance can not be granted. When State or local boards of health consider that the disinfection of a water supply is desirable and wish information upon the subject it will be supplied as fully and freely as possible. All experiments of this kind, however, must be conducted by the board of health, and the Department can serve only in the capacity of an adviser.

COST.

No definite estimate of the cost of the treatment of a reservoir can be given, because of the special conditions governing each case. It is evident, however, that the maximum cost of material for exterminating algæ can not exceed 50 to 60 cents per million gallons, and will often be less than half this amount. The cost for the copper-sulphate destruction of bacteria will be from $5 to $6 per million gallons, and where lime or some soluble hydrate is used in addition the cost would be increased about one-third. The cost of labor necessary to introduce these substances will be slight, since two men can usually treat from 10,000,000 to 20,000,000 gallons in less than three hours.

SUMMARY.

The importance of maintaining all public water supplies at the highest degree of purity and wholesomeness is too well recognized to require any discussion.

The disagreeable odors and tastes so often present in drinking water are due almost exclusively to algæ, although the economic importance of studying these plants has not been recognized until recent years.

These algal forms are widely distributed, and reservoirs in many States have been rendered unfit for use by their presence.

The methods now known for preventing or removing the odors and tastes caused by algæ have proved unsatisfactory, either because of prohibitive expense or failure to accomplish result.

It is therefore desirable that some new, cheap, harmless, and effective method be devised for ridding reservoirs of these pests.

It has been found that copper sulphate in a dilution so great as to be colorless, tasteless, and harmless to man, is sufficiently toxic to the algæ to destroy or prevent their appearance.

The mode of application makes this method applicable to reservoirs of all kinds, pleasure ponds and lakes, fish ponds, oyster beds, water-cress beds, etc. It is also probable that the method can be used for the destruction of mosquito larvæ.

At ordinary temperatures 1 part of copper sulphate to 100,000 parts of water destroys typhoid and cholera germs in from three to four hours. The ease with which the sulphate can then be eliminated from the water seems to offer a practical method of sterilizing large bodies of water, when this becomes necessary.

The use of copper sulphate for the prevention of disease is regarded as incidental and is not designed in any way to supplant efficient preventive measures now in use. It is believed, however, that up to this time no such satisfactory means of thoroughly, rapidly, and cheaply sterilizing a reservoir has been known. Since the selective toxicity of